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ORIGINAL ARTICLE

Association of myeloperoxidase levels with cardiometabolic factors and renal function in prepubertal children

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ABSTRACT

Introduction Myeloperoxidase (MPO), an enzyme linking obesity and cardiovascular (CV) risk in adults, has rarely been studied in young children and no studies assessed its association with renal function. We sought to explore a possible association between serum MPO levels, obesity, CV risk factors and renal function in prepubertal children.

Materials/Methods Cross-sectional evaluation of 309 children aged 8–9 years (161 normal weight, 148 overweight/obese), members of the birth cohort Generation XXI (Portugal). Anthropometrics (body mass index (BMI), waist-to-height ratio (WHtR) and % body fat mass (%BFM) by bioelectrical impedance analysis), 24-h ambulatory blood pressure monitoring and pulse wave velocity (PWV) were measured. Insulin resistance was estimated by the HOMA index (considering serum fasting glucose and insulin determinations). Serum MPO levels were assessed by immunoenzymatic assay.

Results MPO levels were positively associated with obesity indices (BMI *z*-score, WHtR and %BFM). Higher MPO levels were associated with higher 24-h and night-time mean arterial pressure, with nondipping and with higher values of insulin resistance. In normal weight children, the endothelial function, as evaluated indirectly by PWV, was an independent predictor of MPO levels. In overweight/obese children, estimated glomerular filtration rate increased significantly across tertiles of MPO (P_{trend} = 0·031) and this association held after adjustment for age, sex, neutrophil and monocyte counts and CV risk factors.

Conclusions Our results reinforce the role of MPO as a risk marker in obesity and related CV morbidities in young children. MPO levels associate with the dipping pattern and PWV and, among overweight/obese children, an association exists between MPO and renal function.

Keywords Blood pressure, childhood obesity, glomerular filtration rate, myeloperoxidase, pulse wave velocity.

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Introduction

Obesity is a state of low-grade inflammation and associated oxidative stress, which are thought to be key factors in the

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pathogenesis of cardiovascular (CV) diseases and early phases of endothelial dysfunction [1]. Obese children already present a proinflammatory state [2] and increased levels of oxidative stress [3].

Myeloperoxidase (MPO) is an enzyme most abundantly expressed in neutrophils and monocytes that is associated to both inflammation and oxidative stress, as well as linked to the initiation and progression of acute and chronic inflammatory diseases [4]. MPO-derived substances are thought to cause important damage to endogenous cells, especially in the arterial wall, where MPO-catalysed reactions promote the oxidation of low-density lipoprotein (LD) potentially contributing to atherogenesis [5].

In adults, circulating MPO concentrations are higher in obese subjects [6,7], associated with blood pressure (BP) independently of other traditional CV risk factors [8], and predict CV risk [9–11]. Besides, MPO plays a major role in chlorination and nitration of high-density lipoprotein (HDL) in atherosclerotic tissue, depriving HDL of its cardioprotective effects [12]. Elevated MPO levels are also observed in adults with chronic kidney disease (CKD) before and after dialysis initiation [13–15], but the evolution of MPO levels with loss of residual renal function is controversial.

In children, there is little evidence of MPO being associated with CV risk and no study explored its relationship with renal function. Recently, elevated MPO levels were reported in a sample of healthy prepubertal children, in association with obesity and other CV risk factors [16]. Another study found higher MPO concentrations in severely obese as compared to normal weight children, but observed no differences in several macro- and microcirculation parameters at baseline or after a 4-month weight loss programme, although MPO levels decreased [17].

We set out to explore the role of MPO in the context of CV comorbidities in young children. In this study, we aimed to determine whether, in prepubertal children, serum MPO levels are associated with measures of obesity and other CV risk factors. As it is established that obesity generates a milieu favourable to some degree of renal impairment [18], we also aimed to assess the link between MPO and renal function and to determine putative interactions with obesity.

Methods

Study design and sample

We studied children aged 8–9 years that have been followed since birth in a previously established cohort study (Generation 21, Porto, Portugal) [19]. Subjects from the original cohort were eligible for this study protocol (ObiKid project) if they had anthropometric data and a blood sample withdrawn at the 7-year-old evaluation (n = 4590). We aimed to include a minimum sample of 300 children for the Obikid project's main objective, assuming that about 35% would be excluded due to refusal to participate, exclusion criteria or incomplete information, 463 children were preselected to be consecutively screened according to the date of their 7-year-old evaluation: 16 could not be contacted, 32 refused to participate, 23 were unable to schedule the study visits during the recruitment period and 68 met exclusion criteria (four chronic diseases, one chronic usage of medication, 51 living far from the study site and 12 twins). We finally enrolled 324 participants, between August 2013 and August 2014. For the present analysis, 15 children were additionally excluded due to incomplete evaluation, such as absence of blood sample or MPO determination (14), or due to BMI classification as underweight (1). This final sample size (n = 309) provides a statistical power of 86% to detect a difference in MPO levels between normal weight and overweight/obese children of at least 8 ng/mL (assuming SD of 19 and 27 ng/mL in each group, respectively) [20].

Data collection and variable definition

The study visits took place at the Department of Clinical Epidemiology, Predictive Medicine and Public Health, Faculty of Medicine of University of Porto. Anthropometric and general physical examination were performed, according to standard procedures and as previously reported [21]. Waist circumference was indexed to height (waist-to-height ratio, WHtR in cm/m) for statistical analysis. Body mass index (BMI) values were classified according to the World Health Organization reference data for BMI z-score into the following categories: normal weight [> -2 and $\leq +1$ standard deviation (SD)] and overweight/obesity ($>$ 1SD) [22]. In our sample, 88 (28·5%) of 89 (28·8%) children are overweight and 60 (19·4%) of 36 (11·7%) are obese, according to the WHO reference [22] and the IOTF reference [23], respectively. Body fat percentage was assessed by foot-to-foot bioelectrical impedance analysis (Tanita®, model TBF-300, Arlington Heights, IL, USA).

Ambulatory blood pressure monitoring (ABPM) for 24 h was performed in all children with a portable noninvasive oscillometric blood pressure recorder (Spacelabs Healthcare®, model 90207, Snoqualmie, WA, USA). The nondominant arm was used in all children with an appropriate cuff size. BP measurements were taken automatically at 20-min intervals during the daytime and at 30-min intervals during the night-time. A minimum monitoring duration of 24 h with gaps of less than 2 h was required for acceptance; five exams were excluded due to insufficient readings. Hypertension was defined as an average systolic (SBP) and/or diastolic blood pressure (DBP) measurements $\geq$ 95th percentile, during the day or the night, according to the reference values [24]. The absence of dipping pattern was considered as a fall in the MAP during night-time of less than 10% of the corresponding daytime BP.

Carotid-femoral pulse wave velocity (PWV) analysis was performed by a single trained cardiopneumology technician with a portable device (Micro Medical®, model PulseTrace PWV PT4000, Kent, UK); digital volume pulse waveform had to fill 2/3 of the display with little or no noise and artefact to be considered and three measurements of PWV were performed and averaged for analysis. Adequate PWV measurements were available for 308 of the 309 children included in the analysis.

A venous blood sample was collected from all the participants, in the morning period, after an overnight fast of at least 8 h. All the standard laboratory analyses were performed in the Clinical Pathology Department of Centro Hospitalar São João, Porto – Portugal. Insulin resistance was determined using the homoeostasis model assessment index (HOMA-IR) (3 children with missing data). The Zappitelli combined formula was used to estimate GFR (eGFR) [25]. The serum concentration of MPO was assessed in the Department of Pharmacology and Therapeutics of the Faculty of Medicine of the University of Porto by an immunoenzymatic method, using a commercial ELISA kit (BioCheck MPO Enzyme Immunoassay®, Oxis International Inc., Tampa, FL, USA).

Ethics

The ObiKid study was approved by the Ethics Committee of Centro Hospitalar São João, Porto – Portugal and Faculty of Medicine of the University of Porto and it complies with the Helsinki Declaration and the current national legislation. Written informed consent from parents (or their legal substitute) and verbal assent from children were obtained.

Table 1 General characteristics and biochemical parameters by classes of body mass index

	WHO BMI *z*-score classification			
	Normal weight ***n*** **= 161**	**Overweight** ***n*** **= 88**	**Obese** ***n*** **= 60**	***P***
Age (months)	105 ± 3	105 ± 3	106 ± 3	0·458
Male sex	82 (51%)	43 (49%)	39 (65%)	0·114
BMI *z*-score	−0·01 ± 0·70	1·56 ± 0·30	2·66 ± 0·48	< 0·001
WHtR (cm/m)	45 ± 3	50 ± 3	57 ± 5	< 0·001
% body fat mass	10·8 ± 7·2	20·1 ± 7·9	28·1 ± 9·4	< 0·001
24-h mean arterial pressure (mmHg)	81·1 ± 4·4	82·4 ± 5·2	82·6 ± 6·3	0·054
Daytime mean arterial pressure (mmHg)	84·8 ± 4·6	85·7 ± 5·7	86·0 ± 6·5	0·229
Night-time mean arterial pressure (mmHg)	73·3 ± 4·8	74·7 ± 5·2	75·2 ± 6·2	0·026
Absence of dipping pattern	34 (22%)	30 (34%)	16 (27%)	0·104
Pulse wave velocity (m/sec)	4·98 ± 0·49	5·03 ± 0·52	5·20 ± 0·50	0·013
Leucocyte count ($\times 10^9$/L)	6·11 ± 1·75	6·30 ± 1·80	6·55 ± 1·62	0·250
Neutrophil count ($\times 10^9$/L)	2·78 ± 1·35	2·96 ± 1·34	3·12 ± 1·19	0·197
Monocyte count ($\times 10^9$/L)	0·52 ± 0·19	0·54 ± 0·20	0·59 ± 0·18	0·038
Total cholesterol (mg/dL)	157 ± 25	163 ± 28	162 ± 25	0·142
HDL cholesterol (mg/dL)	55 ± 11	54 ± 10	52 ± 9	0·182
Triglycerides (mg/dL)	53 ± 20	62 ± 30	69 ± 33	< 0·001
Fasting glucose (mg/dL)	85·8 ± 5·4	85·4 ± 5·3	87·2 ± 4·9	0·105
Fasting insulin (μIU/mL)	5·7 ± 2·4	7·1 ± 2·9	9·4 ± 5·1	< 0·001
HOMA-IR	1·13 (0·84–1·39)	1·46 (1·07–1·84)	1·68 (1·28–2·64)	< 0·001
High-sensitivity C-reactive protein (mg/L)	0·0 (0·0–0·4)	0·5 (0·2–1·2)	0·8 (0·3–1·9)	< 0·001
Interleukin-6 (pg/mL)	0·75 (0·75–2·43)	1·59 (0·75–3·32)	1·98 (0·75–3·02)	0·069

The values presented are mean ± standard deviation or median (interquartile range), except for sex and the absence of dipping pattern (*n* (%)). The groups of BMI (normal weight, overweight and obesity) were defined according to the WHO classification for BMI *z*-score (19).
BMI, body mass index; WHtR, waist-to-height ratio; HOMA-IR – homoeostasis model assessment of insulin resistance.

Statistical analysis

Data are presented as mean and SD or, if skewed, as median and interquartile range (IQR). MPO had an asymmetric distribution and was logarithmized (base 10) before linear regression analyses, allowing to obtain a normal distribution. Bivariate associations were assessed by Spearman correlation tests. Linear trend was tested using linear regression models with tertiles of MPO and classes of adiposity measures included as independent continuous variables. Linear regression analysis was used to identify independent determinants of MPO and to assess the relationship between MPO and GFR, stratifying by BMI. Reporting of the study conforms to STROBE statement [26,27].

Results

A total of 309 children (53% male) with a mean (SD) age of 8·8 (0·2) years were included in the present analysis. Clinical characteristics and levels of biochemical parameters of all study subjects and separately for normal weight (n = 161) and overweight/obese children (n = 148, of whom 88 overweight and 60 obese) are shown in Table 1.

As shown in Table 2, all measures of obesity (BMI, BMI z-score, WHtR and % body fat mass) significantly increased across tertiles of MPO and older children presented significantly higher levels of MPO. Regarding BP, 24-h and night-time MAP increased across tertiles of MPO and the absence of a dipping pattern was also more frequent among children in the

Table 2 General characteristics and biochemical parameters by tertiles of serum myeloperoxidase

	Serum myeloperoxidase (MPO)			
	1st Tertile [2·55–40·74] ng/mL	2nd Tertile [40·74–72·97] ng/mL	3rd Tertile [72·97–359·15] ng/mL	*P*
Age (months)	105 ± 3	105 ± 3	106 ± 3	0·001
Male sex	58 (56%)	47 (46%)	59 (57%)	0·190
BMI z-score	0·50 ± 1·23	0·82 ± 1·09	1·52 ± 1·17	< 0·001
WHtR (cm/m)	47 ± 5	48 ± 5	51 ± 6	< 0·001
% body fat mass	13·0 ± 10·1	16·5 ± 9·3	21·0 ± 10·5	< 0·001
24-h mean arterial pressure (mmHg)	80·4 ± 4·8	82·7 ± 5·3	82·2 ± 4·9	0·015
Daytime mean arterial pressure (mmHg)	84·4 ± 5·1	86·0 ± 5·5	85·4 ± 5·4	0·173
Night-time mean arterial pressure (mmHg)	72·3 ± 4·8	75·1 ± 5·7	74·8 ± 4·8	0·001
Absence of dipping pattern	16 (16%)	32 (31%)	32 (32%)	0·012
Pulse wave velocity (m/sec)	5·01 ± 0·51	5·03 ± 0·47	5·06 ± 0·54	0·508
Leucocyte count ($\times 10^9$/L)	5·82 ± 1·52	6·15 ± 1·46	6·80 ± 2·05	< 0·001
Neutrophil count ($\times 10^9$/L)	2·49 ± 0·96	2·75 ± 1·00	3·46 ± 1·67	< 0·001
Monocyte count ($\times 10^9$/L)	0·50 ± 0·18	0·52 ± 0·17	0·59 ± 0·21	0·002
Total cholesterol (mg/dL)	161 ± 29	160 ± 26	157 ± 22	0·362
HDL cholesterol (mg/dL)	54 ± 11	55 ± 10	52 ± 9	0·218
Triglycerides (mg/dL)	57 ± 23	59 ± 26	61 ± 30	0·232
Fasting glucose (mg/dL)	86·4 ± 5·5	86·0 ± 5·2	85·3 ± 5·2	0·139
Fasting insulin (μIU/mL)	6·2 ± 3·0	6·9 ± 3·5	7·4 ± 4·0	0·017
HOMA-IR	1·24 (0·97–1·57)	1·26 (0·93–1·85)	1·41 (1·05–1·95)	0·035
High-sensitivity C-reactive protein (mg/L)	0·0 (0·0–0·5)	0·3 (0·0–0·8)	0·4 (0·0–1·1)	0·015
Interleukin-6 (pg/mL)	0·75 (0·75–2·38)	1·73 (0·75–2·42)	1·95 (0·75–3·32)	0·005

The values presented are mean ± standard deviation or median (interquartile range), except for sex and the absence of dipping pattern (n (%)). The tertiles of myeloperoxidase (≤ 40·7; 40·7–73·0; > 73·0) were defined based on all enrolled participants.
BMI, body mass index; WHtR, Waist-to-height ratio; HOMA-IR – homoeostasis model assessment of insulin resistance.

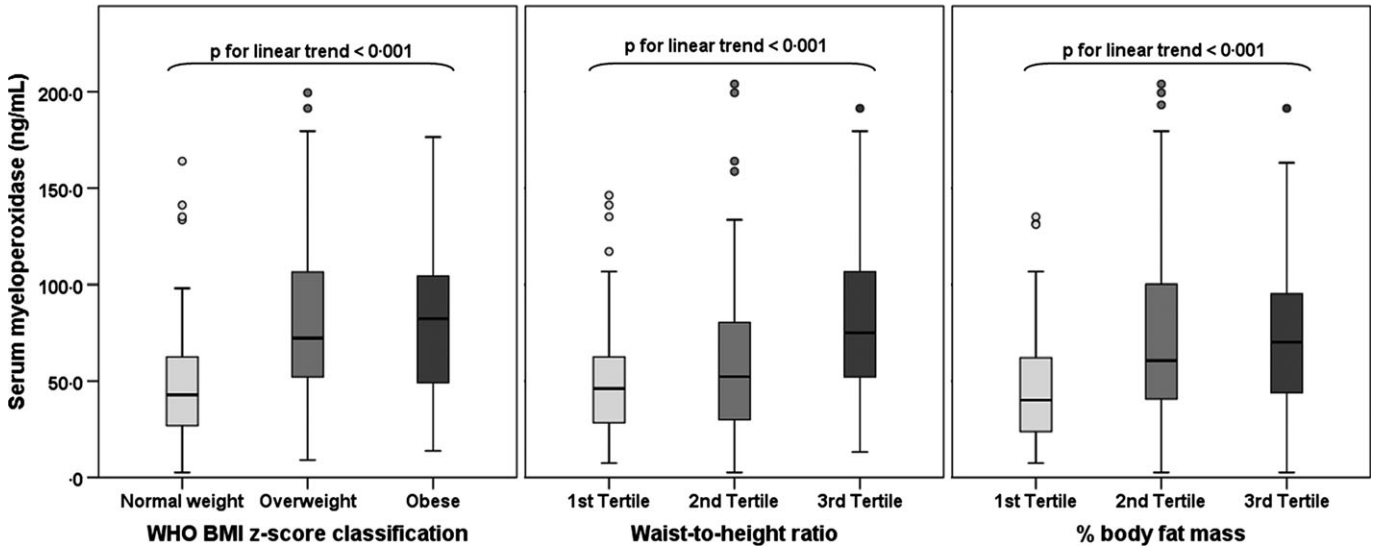

Figure 1 Distribution of serum myeloperoxidase levels by classes of body mass index (BMI) and tertiles of waist-to-height ratio and percentage of body fat mass. The normal weight, overweight and obese group classification is according to the WHO classification for BMI *z*-score (ref. 24). The tertiles of waist-to-height ratio (≤ 45·69; 45·70–50·00; >50·00) and % of body fat mass (≤ 11·43; 11·44–20·70; >20·70) were defined based on all enrolled participants. The myeloperoxidase data are expressed as medians and percentiles 25 and 75. *P* values for linear trend across groups were calculated by linear regression, adjusting for age (in months) and sex.

higher MPO tertiles. Total white blood cell, neutrophil and monocyte counts also significantly increased across tertiles of MPO. Higher levels of hsCRP and IL-6 levels were found in children with higher levels of MPO.

The medians and interquartile range (IQR) of serum MPO levels by classes of BMI and tertiles of WHtR and percentage of body fat mass are shown in Fig. 1. All linear trend analyses across groups were adjusted for sex and age (in months). The normal weight children presented significantly lower levels of MPO than overweight or obese children (42·6 (IQR: 26·8 to 62·2), 72·2 (52·2 to 106·5), 82·4 (48·9 to 105·4) ng/mL, respectively; $P_{trend} < 0·001$). Concordantly, a pattern of increasing median MPO levels across tertiles of WHtR and % body fat mass was found.

In the univariate linear regression analysis, age (in months) and the neutrophil count were associated with serum MPO in the normal weight group, and neutrophil and monocyte counts in the overweight/obesity group (Table 3). After adjusting for all the variables of interest, in the normal weight group MPO increased significantly with age (by +7·5 ng/mL per month of age), with neutrophil count (by +17·0 per 1×10^9/L of neutrophils) and with PWV (by 31·5 ng/mL per m/s of PWV) and decreased significantly with monocytes count (by −72·5 per 1×10^9/L of monocytes). In the overweight/obesity group, the only independent determinant of MPO was the neutrophil count (increase of 10·7 ng/mL in MPO per 1×10^9/L of neutrophils) (Table 3). The effect of age and PWV on MPO levels decreased across BMI classes, being significantly lower in obese compared to normal weight children (*P* for interaction = 0·036 and 0·010, respectively).

In normal weight children, no significant association was found between MPO levels and renal function, whereas in overweight/obese children, levels of eGFR increased significantly across tertiles of MPO (133 (125 to 146), 135 (122 to 142), 135 (126 to 145) mL/min/1·73 m^2, P_{trend} = 0·031) (Fig. 2). This association of MPO levels with eGFR levels proved to be strong and to hold in a fully adjusted multivariate model, with eGFR increasing by 9·9 (95% confidence interval: 0·5 to 19·4, *P* = 0·040) per logarithm of ng/mL of MPO (Table 4).

Discussion

In this study in more than 300 healthy prepubertal children, MPO levels were higher in the presence of obesity, irrespectively of the measure considered. MPO was also associated with other important markers of CV risk, namely the loss of the BP dipping pattern. While in overweight/obese subjects, only the neutrophil count was an independent determinant of MPO levels, in the normal weight endothelial function, as evaluated indirectly by the PWV, was also an independent predictor of the levels of MPO. In addition, we found a strong positive association of MPO with eGFR levels in overweight/obese

Table 3 Mean changes in serum myeloperoxidase per unit of several general and cardiovascular risk variables, in the normal weight and overweight/obesity groups

	Serum myeloperoxidase (ng/mL)							
	Normal weight group				Overweight/obesity group			
	Crude β (95% CI)	*P*	Adjusted β (95% CI)	*P*	Crude β (95% CI)	*P*	Adjusted β (95% CI)	*P*
Male sex (vs. female)	−2·27 (−19·41 to 14·87)	0·794	−4·97 (−22·83 to 12·90)	0·583	−1·48 (−14·83 to 11·88)	0·827	−2·01 (−16·75 to 12·74)	0·788
Age (per month)	4·56 (1·73 to 7·40)	0·002	7·50 (4·19 to 10·81)	< 0·001	1·86 (−0·51 to 4·22)	0·123	2·12 (−0·50 to 4·75)	0·112
24-h MAP (per mmHg)	1·04 (−0·98 to 3·05)	0·311	0·62 (−1·96 to 2·08)	0·514	0·14 (−1·05 to 1·33)	0·815	−0·17 (−1·36 to 1·03)	0·786
Absence of dipping pattern (vs. dipping)	5·84 (−15·43 to 27·10)	0·588	−7·02 (−28·73 to 14·68)	0·523	−1·54 (−15·96 to 12·87)	0·833	−2·63 (−17·13 to 11·87)	0·720
PWV (per m/sec)	13·55 (−3·89 to 31·00)	0·127	31·50 (13·02 to 49·97)	0·001	−0·47 (−13·27 to 12·34)	0·943	−1·07 (−15·09 to 12·94)	0·880
Neutrophil count (per 1 × 10^9/L)	8·30 (2·01 to 14·59)	0·010	17·03 (8·16 to 25·90)	< 0·001	9·67 (4·68 to 14·67)	< 0·001	10·71 (4·00 to 17·43)	0·002
Monocyte count (per 1 × 10^9/L)	5·48 (−40·74 to 51·70)	0·815	−72·49 (−132·98 to −11·99)	0·019	38·81 (4·47 to 73·15)	0·027	−6·89 (−51·00 to 37·23)	0·758
Total cholesterol (per mg/dL)	−0·010 (−0·355 to 0·336)	0·955	−0·056 (−0·489 to 0·376)	0·797	−0·219 (−0·466 to 0·027)	0·081	−0·144 (−0·469 to 0·181)	0·383
HDL cholesterol (per mg/dL)	−0·086 (−0·891 to 0·718)	0·833	−0·026 (−1·066 to 1·013)	0·960	0·020 (−0·670 to 0·710)	0·954	0·351 (−0·512 to 1·214)	0·423
Triglycerides (per mg/dL)	0·168 (−0·264 to 0·601)	0·443	0·106 (−0·374 to 0·587)	0·663	−0·060 (−0·272 to 0·152)	0·578	−0·047 (−0·327 to 0·232)	0·737
Fasting insulin (per μIU/mL)	2·12 (−1·43 to 5·66)	0·240	−2·04 (−11·34 to 7·26)	0·665	−0·07 (−1·70 to 1·57)	0·937	2·30 (−2·37 to 6·97)	0·332
Log-HOMA-IR (per 1 log)	30·61 (−13·94 to 75·17)	0·177	33·57 (−82·91 to 150·05)	0·570	−9·70 (−41·77 to 22·38)	0·551	−57·38 (−148·29 to 33·53)	0·214

The values presented are crude and adjusted linear regression coefficients (β) and 95% confidence intervals. The adjusted β are adjusted for all variables in the table.
24-h MAP, 24-h mean arterial pressure; PWV, pulse wave velocity; Log-HOMA-IR, homoeostasis model assessment of insulin resistance, transformed by logarithm with base 10.

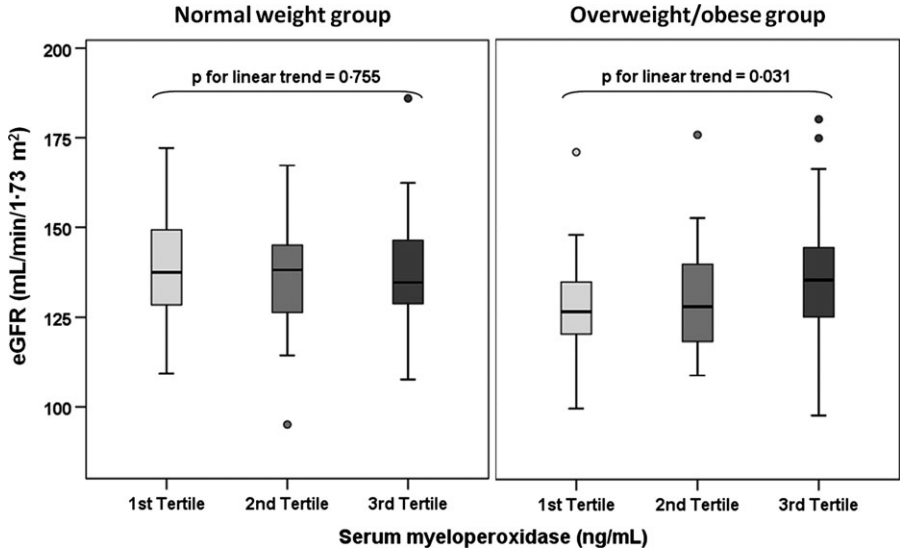

Figure 2 Distribution of estimated glomerular filtration rate by tertiles of serum myeloperoxidase, in the normal weight and overweight/obesity groups. The tertiles of myeloperoxidase (≤ 40·7; 40·7–73·0; > 73·0) were defined based on all enrolled participants. The eGFR data are expressed as medians and percentiles 25 and 75. The value of *P* for linear trend was estimated by linear regression analysis of eGFR levels on MPO levels, adjusting for age (in months) and sex. The normal weight group includes only children with normal weight, and the overweight/obesity group includes children with overweight or obesity, according to the WHO classification for BMI *z*-score [22]. eGFR, estimated glomerular filtration rate by Zappitelli combined formula.

Table 4 Mean changes in estimated glomerular filtration rate per logarithm of serum myeloperoxidase in the overweight/obesity group

	eGFR (mL/min/1·73 m²)	
	β (95% CI)	*P*
MPO (crude)	11·0 (2·2 to 19·9)	0·015
MPO + age (months) + sex	10·8 (1·8 to 19·8)	0·019
Fully adjusted model*	9·9 (0·5 to 19·4)	0·040

The values presented are linear regression coefficients (β) and 95% confidence intervals, estimated by multiple linear regression, with eGFR (estimated glomerular filtration rate by Zappitelli combined formula) as dependent variable and serum myeloperoxidase (transformed by logarithm with base 10) as independent variable, adjusting for several variables as stated.

*The fully adjusted model is adjusted for sex and age (in months), BMI *z*-score, hypertension (defined as an average systolic and/or diastolic blood pressure measurements ≥ 95th percentile, either during the day or the night, according to Urbina *et al.* reference tables [24]), pulse wave velocity, neutrophil count, monocyte count, insulin resistance by homoeostasis model assessment (transformed by logarithm with base 10) and HDL cholesterol.

children, which held after adjustment for several CV risk factors.

The finding that MPO levels associate with obesity is well established in adults [6,7]. Our findings that MPO levels are higher in overweight and obese children and increase across WHtR and % body fat mass tertiles are in accordance with the few studies that exist in children. Two recent studies in prepubertal children and another in adolescents reported that MPO, considered as a biomarker of CVD, was significantly higher in the obese children [17,20,28]. Another study found increased levels of MPO and others markers of oxidative stress, inflammation and endothelial activation in obese children with vitamin D insufficiency [29]. hsCRP is a biomarker of CV risk in adults and both hsCRP and IL-6 are known to interfere with endothelial function, in part by stimulating inflammation-oxidative stress pathways [1]. In our study, both hsCRP and IL-6 levels increased across tertiles of MPO, which is concordant with the positive significant correlations found by Olza *et al.* [16].

MPO is regarded as a common link between inflammation and oxidative stress systems and closely related to the vascular wall. In our study, we observed that higher MPO levels are associated with increasing levels of 24-h and night-time BP and with the loss of the dipping pattern. To our knowledge, no previous studies addressed the association of MPO levels and ABPM as completely as we have done. Evidence supports that, even in children, ABPM is more accurately related with target-organ damage and is a better predictor of CV risk than office BP measurements [30,31]. Also, in adults, the nondipping pattern seems to increase the risk of BP-related complications and overall mortality [32]. However, the prevalence of nondipping in the general paediatric population before the onset of

hypertension or in the setting of obesity is largely unknown. A review from 2009, states that, in children, BMI correlates with ABPM, especially with systolic and night-time BP, but that the dipping is not consistently affected across studies [33]. The physiological mechanisms behind loss of dipping are still unclear, but it is hypothesized that it might be related to changes in insulin–glucose metabolism. In fact, in adults, MPO was positively and independently associated with office SBP and DBP and the association was stronger in the presence of hyperglycaemia or oxidative stress [8]. In another study in adults, a specific antihypertensive treatment reduced both ABPM and inflammatory and oxidative biomarkers, including MPO [34]. However, previous studies in young children failed to find an association between dipping and insulin resistance [35,36]. Accordingly, in our study, we found no association between glucose, insulin or HOMA-IR levels and the dipping pattern (data not shown).

Childhood BP tracks into adult life and represents one of the most important predictors of CV risk in later life [37]. Thus, the fact that prepubertal overweight/obese children with higher levels of MPO already had higher night-time BP raises the possibility that, at this age, MPO-derived reactive substances may already be associated with injury to the arterial wall. Our novel finding that in normal weight children MPO levels were independently associated with PWV reinforces this hypothesis, suggesting that MPO might contribute to vascular stiffness and atherogenesis, even at prepubertal age. The capacity of MPO to reduce the availability of the endogenous vasodilator nitric oxide and the possible interference with lipids in the vascular wall have been proposed as possible mechanisms of injury [12,38,39]. Although a recent study found no differences in brachial artery or microvascular reactivity in severely obese children with higher levels of MPO, PWV was not evaluated [17]. In addition, we found that most of the independent determinants of MPO in normal weight children were not significant in the overweight/obesity subgroup of our cohort, suggesting that obesity, *per se*, is an important and strong determinant of MPO. Actually, there were significant interactions between BMI *z*-score categories and both age and PWV, with the effect of these variables decreasing across BMI classes. While insulin and HOMA-IR did not independently predict MPO, this might be explained by the fact that only few children in our sample were insulin-resistant by the HOMA-IR criterion.

Our findings concerning the association of MPO with renal function in overweight/obese children were somewhat unexpected. It is well established that obesity initially causes physiological (mal)adaptation of the kidney with hyperfiltration and hyperperfusion consequently leading to proteinuria and progressive renal damage [40]. We speculate that the overweight/obese children with higher GFR in our cohort might be subject to such glomerular hyperfiltration, and MPO might be directly involved in this process. *Ex vivo* perfusion of glomeruli with MPO and H_2O_2 has been demonstrated to induce proteinuria, endothelial cell swelling, and epithelial cell foot process effacement [41] and local MPO activity contributes to glomerular damage in experimental glomerulonephritis [42].

Strengths and limitations

Our study covers a novel area of investigation and adds important findings regarding MPO as a biomarker of long-term CV risk in young children. The strengths of our study are the large sample of prepubertal children, homogenous in terms of age, and the extensive evaluation of inflammation and CV risk markers. The study of this complex interplay of systems in young individuals avoids the issue of secondary pathology related to target-organ damage and comorbidities often encountered in ageing populations. The availability of ABPM and PWV data in all children is an additional major strength and novelty of this study, as associations of MPO with these of CV health indicators have rarely been assessed before and not at all in young children. The assessment of the association of MPO with renal function in children is another innovative facet of our study.

Our study is limited by its cross-sectional design. Only a prospective evaluation could provide unequivocal evidence on cause–effect relationships and the usefulness of MPO as a biomarker of emerging CV morbidity.

Conclusion

In this study, we demonstrate that MPO levels are associated with nocturnal BP dipping and PWV, and that, among overweight/obese children, an association exists between MPO levels and renal function. Therefore, the role of MPO as a relevant pathogenic factor and as a biomarker of risk in obesity-related CV morbidities is reinforced, by showing that young overweight and obese children already present an inflammatory and pro-oxidant milieu that may contribute to endothelial impairment. Nevertheless, the mechanisms and the causal associations involved continue to be poorly understood. More studies are needed to enlighten the role of oxidative stress and inflammation in the pathophysiology of disease, especially in children, ideally with long-term follow-up studies.

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Conflict of interests

The authors have no conflict of interests to disclose.

Contributions

Liane Correia-Costa, Teresa Sousa, Alberto Caldas Afonso, António Guerra, Henrique Barros and António-Albino Teixeira and Ana Azevedo conceived and designed the present study and were responsible for all the field work, as well as the data analysis and interpretation. Franz Schaefer, Cláudia Moura, Cláudia Mota and José Carlos Areias collaborated in the data analysis and interpretation and performed literature research. Manuela Morato, Dina Cosme and Joana Afonso carried out experiments and were involved in data analysis and interpretation. All authors were involved in writing the manuscript and had final approval of the submitted and published versions.

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